For life science research only. Not for use in diagnostic procedures.

DOTAP Liposomal Transfection Reagent

N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate

Version: 14

Content Version: March 2021

For the cationic liposome-mediated transfection of eukaryotic cells

Cat. No. 11 202 375 001	2 ml 5 x 400 µl, 5 x 400 µg

Store the reagent at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DOTAP Liposomal Transfection Reagent	Aqueous dispersion (liposomes) in MBS (MES-buffered saline, pH 6.2), 1 mg/ml. ▪ Bottled under argon. ▪ Filtered through 0.2 µm pore-size membrane.	5 vials, 400 µl, 400 µg each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the reagent is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	DOTAP Liposomal Transfection Reagent	Store at +2 to +8°C. The reagent can be repeatedly used for up to 2 months.

1.3. Additional Equipment and Reagent required

For Transfection

- HBS (HEPES-buffered saline)
- Nucleic acid stock solution in storage buffer
- Culture medium with or without serum

i *For additional information about these reagents, see section,* **Working Solution.**

- Polystyrene or glass tubes

For Optimization of Transfection Conditions

- CAT ELISA*
- β-Gal ELISA*
- hGH ELISA*
- X-Gal*

For Preparing Adherent Cells

- Trypsin
- Dispase*

For Stable Transfections

- G-418 Solution*
- Hygromycin B*

1.4. Application

DOTAP Liposomal Transfection Reagent is a liposome formulation of the cationic lipid DOTAP. It can be used for the:

- Highly efficient transfection of DNA, including yeast artificial chromosomes (YACs) into eukaryotic cells for transient or stable gene expression.
- Efficient transfer of other negatively charged molecules, such as RNA, oligonucleotides, nucleotides, proteins, and ribonucleoprotein (RNP) complexes into eukaryotic cells.

Mixing DOTAP with DNA results in spontaneously formed stable complexes. These complexes can be added directly to the tissue culture medium. This method of DNA transfer is very gentle, avoiding the cytotoxic effects commonly associated with lipofection or other transfection methods. Unlike other liposomal reagents, DOTAP produces highly efficient transfections both in the presence and absence of serum. It can also be successfully used for *in vivo* applications.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Purity of Nucleic Acids

The nucleic acid (DNA or RNA) to be transfected must be highly purified by, for example, column chromatography or cesium chloride gradient centrifugation, and be free of traces of residual cesium chloride to avoid possible cytotoxic effects.

Cell Lines

Independent of the transfection method used, the success of a particular transfection can vary substantially with the cell line and the state of the individual cells used. Reproducible, successful transfections require careful optimization and standardization of the transfection conditions for each cell type. Cells used for transfection experiments must be healthy, well proliferating, and plated at a constant density to minimize both the intra- and interassay variance. Therefore, we recommend using cells that have been regularly passaged, to ensure that they are in a permanent growth phase. We also recommend subculturing the cells the day before performing the actual transfection experiment
(see section, **Preparation of Cells**).

Recommended Culture Media

DOTAP Liposomal Transfection Reagent differs from other cationic liposome formulations for transfection by producing highly efficient transfections both in the presence and the absence of serum. Therefore, in most cases, the type of culture medium used for routine culture of the cells, such as serum-containing medium, should also be used during transfection.

Optimization of Transfection Conditions

To establish optimal transfection conditions, determine the expression level of a reporter protein by varying experimental parameters as described below, using, for example, the CAT ELISA* (see Figure 1), β-Gal ELISA*, or the hGH ELISA*, depending on the reporter system used. Alternatively, monitor transfection efficiency using, for example, histochemical staining of cells transfected with a lacZ construct with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal)*.

Working Solution

Prepare the following solutions prior to transfection.

Reagent	Preparation
HBS	▪ 20 mM HEPES (cell culture grade), 150 mM NaCl, pH 7.4. ▪ Dissolve 876 mg NaCl in 90 ml double-distilled water. Add 2 ml, 1 M HEPES and adjust the pH to 7.4. Make up to 100 ml and filter through a 0.2 µm pore-size membrane.
Nucleic acid stock solution in storage buffer	Commonly used storage buffer: 10 mM Tris-HCl, 1 mM EDTA; pH 7.4, filtered through a 0.2 µm pore-size membrane before lyophilization.
Culture medium	With or without serum, filtered through a 0.2 µm pore-size membrane.

2.2. Protocols

Preparation of Cells

Adherent Cells

1. The day before transfecting the cells, subculture cells according to standard protocols.

2. Resuspend cells mechanically in the case of loosely attached cells, or enzymatically, using, for example, a Trypsin or Dispase* solution.

3. When replating cells, choose a plating density yielding approximately 60 to 80% confluency at the time of the transfection.
   - Depending on the particular cell's shape and its proliferative activity, the number of cells to be plated can vary within the range depicted in the **Experiment Overview**.
   - The correct choice of an optimal plating density also depends on the planned time interval between transfection and reporter gene analysis. For a large interval, we recommend a lower density, whereas a somewhat higher density may be beneficial for a short interval.

Suspension Cells

Passage the cells the day before transfection to a density of 1 to 3 × 10^5 cells/ml in a final volume of approximately 5 ml of fresh culture medium (see section, **Experiment Overview**).

Preparation of the DOTAP/Nucleic Acid Mixture

The following is an example of the transfection of adherent and suspension cells with DNA for cells cultured in a 6 cm diameter culture dish. See the **Experiment Overview** for required reaction components for other dish sizes. Transfection of cells with RNA is performed following an identical protocol, replacing DNA with RNA. Depending on the particular cell type and DNA (RNA) construct to be transfected, choose a ratio of 1 µg DNA (RNA) per 5 to 10 µl DOTAP.

⚠ ***To obtain optimal transfection efficiencies, use polystyrene or glass tubes.***

Follow the steps below for a standard application.

1. In a autoclaved reaction tube, dilute 5 µg DNA (RNA) to a concentration of 0.1 µg/µl in HBS buffer (final volume 50 µl).

2. In a separate autoclaved reaction tube, mix 30 µl DOTAP with HBS buffer to a final volume of 100 µl.

3. Transfer the nucleic acid solution from Step 1 (50 µl) to the reaction tube already containing the DOTAP in HBS buffer from Step 2 (100 µl).
   - Carefully mix the transfection mixture by gently pipetting the mixture several times.

   ⚠ *Do not vortex or centrifuge.*

4. Incubate the transfection mixture for 10 to 15 minutes at +15 to +25°C.
   - The occurrence of clouding in the reaction mixture does not affect transfection efficiency.

Transfection

Adherent Cells

1. Gently mix the DOTAP/nucleic acid mixture, prepared as described in section, **Preparation of the DOTAP/ Nucleic Acid Mixture** with 5 to 6 ml culture medium.
   - Use the DOTAP/nucleic acid mixture at a final concentration not exceeding 30 µg DOTAP per ml culture medium.

2. Remove culture medium and add the culture medium containing the DOTAP/nucleic acid mixture to the culture dish.
   - Alternatively, the DOTAP/nucleic acid mixture may be added directly to the cultures and gently mixed, or by rocking the culture dish to ensure an equal distribution of the transfection mixture.

3. Incubate cells with the DOTAP/nucleic acid mixture for approximately 3 to 6 hours (see section, **Incubation Time**).

4. Replace medium with fresh culture medium and further incubate cells.

5. Determine transfection efficiency using, for example, the nonradioactive CAT ELISA*, β-Gal ELISA*, or hGH ELISA*. Detailed information on the methodology is given in the respective Instructions for Use.

Suspension Cells

1. Gently mix the DOTAP/nucleic acid mixture, prepared as described in section, **Preparation of the DOTAP/ Nucleic Acid Mixture** with 5 to 6 ml culture medium. Use the DOTAP/nucleic acid mixture at a final concentration not exceeding 30 µg DOTAP per ml culture medium.

2. Centrifuge suspension cells for 10 minutes at 250 × *g* using a autoclaved centrifuge tube.
   - Remove supernatant and resuspend cells in culture medium containing DOTAP/nucleic acid mixture.
   - Transfer cells into an appropriate culture flask (7.5 ml) or culture dish (6 cm).
   - Alternatively, the DOTAP/nucleic acid mixture may be added directly to the cultures and gently mixed, or by rocking the culture flask or dish to ensure uniform distribution of the transfection mixture.

3. Incubate cells with the DOTAP/nucleic acid mixture for approximately 3 to 6 hours (see section, **Incubation Time**).

4. Replace medium with fresh culture medium and incubate cells.

5. Determine transfection efficiency using, for example, the nonradioactive CAT ELISA*, β-Gal ELISA*, or hGH ELISA*. Detailed information on the methodology is given in the respective Instructions for Use.

Transfection in Serum-Free or Serum-Reduced Culture Media

1. Gently wash cells 3 times with serum-free or serum-reduced culture medium.
   - For suspension cells, centrifuge cells 3 times for 10 minutes at 250 × *g* using a autoclaved centrifuge tube, replacing the supernatant after each centrifugation step with fresh culture medium.

2. After the last washing, add fresh culture medium containing the DOTAP/nucleic acid mixture and proceed as described in section, **Transfection**.

If transfecting cells routinely cultured in serum-containing media under serum-free or serum-reduced conditions, preculturing washed cells in the culture medium used for transfection for 1 to 5 hours prior to the addition of the DOTAP/nucleic acid mixture may increase transfection efficiency.

Incubation Time

Typically, incubation of cells to be transfected with the DOTAP/nucleic acid mixture for 3 to 10 hours is sufficient to yield good transfection efficiencies. However, cells may be cultured in culture medium containing the DOTAP/ nucleic acid mixture for up to 72 hours with no cytotoxic side effects. With very sensitive or rapidly proliferating cells, the addition of fresh culture medium or total replacement with fresh culture medium post transfection can have a favorable effect.

Stable Transfection

Transfection of eukaryotic cells, with the goal to produce stably transformed cells, is performed following the protocols in sections, **Preparation of Cells** and **Transfection**. Here, plate cells at a somewhat lower density, yielding approximately 50% confluency at the time of transfection and adding the DOTAP/DNA mixture in fresh culture medium. Use a culture medium containing serum.
Subsequent to transfection, the culture medium containing the DOTAP/DNA mixture is replaced with a selection medium containing a selection antibiotic. Commonly used selection antibiotics are Geneticin (G-418 Solution)* and Hygromycin B*. Detailed information on establishing the required concentration of the respective antibiotic and performing the selection and cloning of stably transfected cells is given in the respective Instructions for Use.

Optimization of Transfection Conditions

Replacement of the culture medium prior to transfection is not required, but may increase transfection efficiency, depending on the particular application. Transfection efficiencies may vary with the cell type used. To obtain optimal results, the following parameters may be modified:

Parameter	Solution
Incubation time	3 to 10 hours yields good results, however the cells may be incubated with the DOTAP/nucleic acid mixture for up to 72 hours without cytotoxic effect.
Amount of DOTAP	5 to 10 µl per µg DNA (RNA). The final concentration of DOTAP should not exceed 30 µl/ml culture medium.
Amount of DNA or RNA	May be optimized (*e.g.*, in the range of 1 to 5 µg DNA (RNA) for a 60 mm culture dish). If an amount of DNA (RNA) above 5 µg is to be used, increase the volume of DOTAP and HBS buffer accordingly.

Experiment Overview

1. Subculture cells the day before transfection.

	Adherent Cells			Suspension Cells
Diameter of culture dish	35 mm	60 mm	100 mm	-
Cell number yielding 60 to 80% confluency	1.0 - 3.0 × 10^5/dish	0.5 - 1.0 × 10^6/dish	1.5 - 2.5 × 10^6/dish	1.0 - 3.0 × 10^5/ml
Volume of culture medium	1 - 2 ml	5 - 6 ml	10 - 14 ml	2 - 5 ml

2. Prepare the DOTAP/DNA mixture.

	Adherent Cells			Suspension Cells
Diameter of culture dish	35 mm	60 mm	100 mm	-
2a - Dilute DNA to a final volume of:	≈ 2.5 µg/25 µl	≈ 5 µg/50 µl	≈ 7.5 µg/75 µl	≈ 5 µg/50 µl
2b - Dilute DOTAP to a final volume of:	15 µl/50 µl	30 µl/100 µl	45 µl/150 µl	30 µl/100 µl

- Mix the DNA (Step 2a) and DOTAP (Step 2b) solutions and incubate 10 to 15 minutes at +15 to +25°C.

Dilute DNA and DOTAP in HBS buffer as described in the protocols.

When using an amount of DOTAP different from that given in (2b), modify the volume of HBS buffer accordingly. For example, when doubling the amount of DOTAP from 15 to 30 µl, also double the final volume from 50 to 100 µl (see Figure 1).

3 Replace culture medium with fresh medium containing the DOTAP/DNA mixture.
- Transfect adherent and/or suspension cells for 3 to 10 hours at +37°C.

4 Transfected cells can now be used for downstream applications.
Maintain cells at +37°C at all times.

2.3. Parameters

Chemical Formula

$C_{43}H_{83}NO_8S$

Chemical Name

N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP)

Chemical Structure

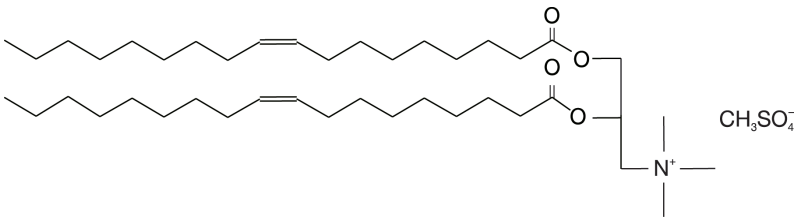

Fig. 2: Chemical structure of DOTAP.

Molecular Weight

774.19 Da

Purity

>99% (elementary analysis, TLC)

Toxicity

Cytotoxicity

In experiments with peripheral blood lymphocytes and HeLa cells, DOTAP Liposomal Transfection Reagent was shown to be non-cytotoxic below a concentration of 150 µg/ml.

Working Concentration

Use approximately 5 to 10 µg/ml per µg DNA. The optimal working concentration is dependent on several parameters, including the cell line and nucleic acid being used and the incubation time. It is important to optimize the transfection conditions for the individual cell type studied (see section, **Protocols**).

3. Results

Transfection of HeLa cells in culture medium with 10% FBS (fetal bovine serum) with plasmid pSV2-CAT. Determination of CAT expression with the CAT ELISA*.

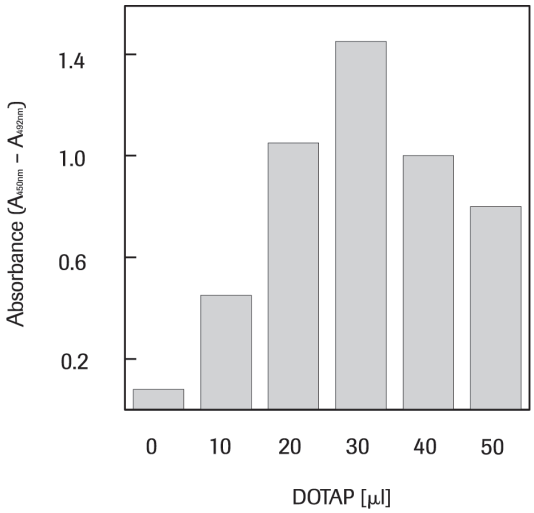

Fig. 1: Determination of CAT expression. 1.0 × 10^6 HeLa cells in 6 cm Petri dishes were transfected with 5 µg pSV2-CAT and varying amounts of DOTAP (1 mg/ml) (0 – 50 µl) in 6 ml of culture medium containing 10% FCS. The cells were incubated for 6 hours. The medium was aspirated, new culture medium was added, and the cells were incubated for an additional 40 hours. Cells were lysed and CAT expression was analyzed using the CAT ELISA.

4. Additional Information on this Product

4.1. Test Principle

A number of methods have been developed to transfer DNA into eukaryotic cells for the study of gene regulation and gene expression. These methods include the use of calcium phosphate or other divalent cations, polycations, liposomes, retroviruses, microinjection, and electroporation. However, all of these methods suffer from one or more problems related to cellular toxicity, poor reproducibility, inconvenience, or inefficiency of DNA delivery. An important addition to the repertoire of DNA-transfection methodologies is cationic liposome-mediated transfection (lipofection). DOTAP Liposomal Transfection Reagent has been proven to be a highly effective tool for the transfection of DNA, RNA, oligonucleotides, ribonucleoprotein particles, and proteins, and helps to avoid the problems commonly associated with transfection.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
Typing error in section **Experiment Overview** (2b) has been corrected.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Collagenase/Dispase®	100 mg	10 269 638 001
	500 mg	11 097 113 001
Dispase® II (neutral protease, grade II)	5 x 1 g	04 942 078 001
Dispase® I (neutral protease, grade I)	10 x approx. 2 mg, ≥ 20 U	04 942 086 001
X-Gal	100 mg, *Not available in US*	11 680 293 001
	100 mg	03 117 073 001
	250 mg	10 651 745 001
	1 g	10 745 740 001
	2.5 g	10 703 729 001
Hygromycin B	1 g, 20 ml	10 843 555 001
hGH ELISA	1 kit, 192 tests	11 585 878 001
CAT ELISA	1 kit, 192 tests	11 363 727 001
G-418 Solution	20 ml, 1 g	04 727 878 001
	100 ml, 5 x 20 ml	04 727 894 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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